



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: AGENTS AND METHODS FOR THE CONTROL OF FUNGAL AND BACTERIAL DISEASES			
(57) Abstract			
<p>A method and agents for the control of fungal and bacterial diseases are provided. In one embodiment, specific chelates of one or more metal ions operate to control bacterial caused diseases in plants. In another embodiment, fungal disease in plants is controlled by employing a chelate of zinc ion, together with a chelate of another metal. Some of the metals preferred for use in combination with zinc comprise copper and/or manganese. Some preferred chelating agents comprise glycine, citric acid or resorcinol. The chelates may be based upon organic acids, organic alcohols or amines, aromatic ring substitution products, or other synthetic chelating agents combined with a metal.</p>			

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## AGENTS AND METHODS FOR THE CONTROL OF FUNGAL AND BACTERIAL DISEASES

### FIELD OF THE INVENTION

This invention relates generally to disease control. More specifically, the  
5 invention relates to the control of diseases caused by fungi or bacteria. Most  
specifically, the invention relates to methods and materials for controlling fungal or  
bacterial disease in plants.

### BACKGROUND OF THE INVENTION

Fungal diseases are very common in crops in various stages of the  
10 growth cycle, sometimes with devastating effect. Commercially there are many  
control agents to combat such diseases, belonging to diverse chemical groups.

IL 97676 discloses compositions and a method for the control of fungal  
diseases using citric acid chelates of copper, zinc, manganese and calcium either  
15 singly or in combination. The specific identity of the diseases was not disclosed  
apart from the fact that the pest was a fungus in each example given.

The Pesticide Manual (Ed. C.R. Worthing, The British Crop Protection  
Council Ninth Edition, 1991), lists several commercial fungicides which are  
complexes of thiocarbamates with one of the following metals: manganese, zinc,  
iron, copper or specific mixtures of such complexes. The carbamates *per se* are  
20 well known fungicides and are used in that sense also without the addition of  
metal.

Bacterial diseases occur in many crops in various stages of the growth  
cycle, sometimes with devastating effect, such as fireblight in pears and apples.

Some storage or "postharvest" diseases of agricultural products are caused by bacteria.

Commercially there are not many control agents to combat such diseases, the most prominent ones are antibiotics and copper based control  
5 agents.

## SUMMARY OF THE INVENTION

It is an object of the invention to provide a composition for controlling fungal and bacterial diseases in plants.

According to the invention there is provided a composition for the control of fungal diseases which contains at least one type of chelating agent complexed with zinc ion and with at least another metal ion, whereas the chelating agent's molecules are characterized in being either synthetic or natural.

According to the invention, bacterial pathogens can be controlled by treating the infected plants or cultures of the bacteria with chelated metal ions, most notable in this respect is zinc. However, other chelated metals are active singly, or in mixture with chelated metals, notably with chelated zinc.

A second aspect of the invention is that a certain bacterial pathogen tends to be more susceptible to a specific chelating agent or a combination of such agents in connection with a certain metal or with a combination of such metals, than other bacterial pathogens.

In one specific embodiment, specific chelates of one or more metal ions operate to control bacterial caused diseases in plants. These chelates may be based upon organic acids, organic alcohols or amines, aromatic ring substitution products, or other synthetic chelating agents combined with a metal, which may comprise zinc, copper, or manganese, used either singly or in combination. In another aspect of the present invention, it has been found that fungal disease in plants can be effectively controlled by employing a chelate of zinc ion, together with a chelate of another metal. Some of the metals preferred for use in

combination with zinc comprise copper and/or manganese. Some preferred chelating agents comprise glycine, citric acid or resorcinol.

These agents are highly effective in controlling both bacterial and fungal diseases in plants.

## DETAILED DESCRIPTION OF THE PRESENT INVENTION

Fungal Infestation**Preparation of Compositions**

Following are various compositions (termed henceforth "test  
5 compositions") that were tried in various experiments, for their effect on fungal  
pathogens. All preparatory steps were carried out accompanied by occasional  
stirring, until a clear solution was obtained in each case.

**i) Citric Acid Chelates**

Composition A: a measured amount of copper (supplied as  
10  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), was thoroughly mixed in water with equimolar amount of citric  
acid, then water added to form a final 0.83 M copper in solution.

Composition B: a measured amount of manganese (supplied in the form  
of  $\text{MnSO}_4 \cdot 1\text{H}_2\text{O}$ , was thoroughly mixed in water with equimolar amount of citric  
acid, then water added to form a final 1.65 M manganese in solution.

15 Composition C: a measured amount of zinc (supplied in the form of  
 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ), was thoroughly mixed in water with equimolar amount of citric  
acid, then water added to form a final 1.5 M zinc in solution.

Composition D: an equal volume mixture of composition A and B. Final  
concentration of total metals: 1.2 M

20 Composition E: an equal volume mixture of composition A, B and C.  
Final concentration of total metals: 1.3 M.

## ii) Amino Acid Chelates

Composition F: a measured amount of copper (supplied in the form of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), was thoroughly mixed in water with equimolar amount of glycine, then water added to form a final 1.19 M copper in solution.

5        Composition G: a measured amount of zinc (supplied in the form of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ), was thoroughly mixed in water with equimolar amount of glycine, then water added to form a final 1.6 M zinc in solution.

Composition H: an equal volume mixture of composition G and F. Final concentration of total metals: 1.3 M.

10

## Examples

Fungal growth was determined by measuring radial size of culture growing in plates on potato dextrose agar. Test solutions at various concentrations were incorporated into plates seeded with 5 mm plug of one week  
15    old cultures. Colony diameter was determined at various times of incubation at 25°C.

**Example 1.** *Penicillium digitatum*, a common fungal pest, was treated in plates as described above, results are shown in Table 1.



**Table 1: Colony Size (in cm) After Incubation with Various Concentrations  
(% in growth medium) of the Test Compositions.**

Test Composition	0 %	1.0 %	2.5 %
A	3.5	0.0	0.0
B	3.5	2.0	1.63
D	4.5	2.0	1.2
E	3.5	0.0	0.0

In this example, manganese chelate, and mixed copper and manganese chelates were least effective. The copper chelate and the mixed zinc copper and manganese chelates were much more effective.

**Example 2.** *Botrytis cynerea*, a common fungal pest, was treated in plates as described above, results are shown in Table 2.

**Table 2: Colony size (in cm) after Incubation with Various Concentrations  
(% in growth medium) of the Test Compositions.**

Test Composition	0 %	1.0 %	2.5 %
A	9.0	8.0	0.27
B	9.0	9.0	7.6
D	9.0	9.0	9.0
E	9.0	2.4	1.9

In this example, as in the former one, manganese chelate, and mixed copper and manganese chelates were least effective. The mixed chelate of zinc, copper and manganese was most potent in the lowest effective concentration (1%), although the copper chelate had a better effect in the higher concentration (5%).

**Example 3.** Visual observation was used to find the inhibitory concentration of test compositions. Tissue culture wells were used to grow cultures of the fungi on agar, each consecutive well in a row containing an increasing concentration of the test composition. Assessment of inhibitory concentration of the test composition was done under the microscope.

**Table 3a: Inhibitory Concentration Of Test Compositions (In % Of Culture Medium) On *Botrytis Cynerea* Cultures**

Inhibition marks: +++ no inhibition; ++ 40-70% inhibition; + 80-90 % inhibition; - total inhibition.

Test Composition	0 %	0.1 %	0.2% .	0.5 %
F	+++	+++	++	-
G	+++	+++	+	-
H	+++	++	-	-

**Table 3b: Inhibitory Concentration of Test Compositions (in % of Culture Medium) on *Penicillium Expansum* Cultures**

Inhibition marks: +++ no inhibition; ++ 40-70% inhibition; + 80-90 % inhibition; - total inhibition.

Test Composition	0 %	0.1 %	0.2%	0.5 %
F	+++	+++	+	-
G	+++	+++	++	-
H	+++	++	-	-

**Table 3c: Inhibitory Concentration of Test Compositions (in % of Culture Medium) on *Penicillium Digitatum* Cultures**

Inhibition marks: +++ no inhibition; ++ 40-70% inhibition;

5 + 80-90 % inhibition; - total inhibition.

Test Composition	0 %	0.05%	0.1 %	0.2%	0.5 %
F	+++	++	+	-	-
G	+++	+++	++	+	-
H	+++	++	-	-	-

The results show that for each of the three above well known plant pests, no matter whether the copper chelate or the zinc chelate alone were more potent,  
 10 it was the mixture of the two which was most effective.

### **Bacterial Infestation**

Following are various compositions (termed henceforth "test compositions") that were tried in various experiments, *in vivo* and or *in vitro* for their effect on bacterial pathogens. All preparatory steps were carried out  
 15 accompanied by occasional stirring, until clear solution was obtained.

Composition A: 223 g citric acid , 94 g zinc oxide 450 ml water, then 50 ml nitric acid and 50 ml sulfuric acid, mixed and water added to a final volume of 700 ml.

Composition B: 223 g citric acid, 290 g cupric sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), to  
 20 that about 1200 ml water added to a final total volume of 1400 ml.

Composition C: an equal volume mixture solution of compositions A and B.

Composition D: 223 g citric acid, 196 g manganese sulfate ( $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ ), to that water added to a final volume of 800 ml.

5      Composition E: an equal volume mixture solution of A, B and D.

Composition F: 10 g glycine, 38.6 g cupric sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), water added to a final volume of 132 ml.

Composition G: 28 g glycine, 30 g zinc oxide, 100 ml water, 25 ml sulfuric acid, then water added to a final volume of 235 ml.

10      Composition H: 80% concentration of composition E.

Composition I: 30 g ZnO, 200 water, 30 ml sulfuric acid, 41 g resorcinol, water added to a final total volume of 340 ml.

Composition J: an equal volume mixture solution of F and G.

### Field experiment 1: Effectivity of Composition in an Orchard

15      Pears are very susceptible to *Erwinia amylovora* attack, causing the trees a severe disease called fireblight, the symptoms of which are blackening and death of twigs and branches, with gradual progression towards the trunk, often leading to death of whole trees and entire orchards. As of today, there are no known efficient cures for the disease. An experiment was designed to assess the

20      efficacy of a composition according to the invention against an attack by the bacterial pathogen.

The experiment was carried out in a mature pear orchard of the variety Spadona, in northern Israel. Plot size of four trees were employed for each replication, with four random placed replications of each treatment.

First spraying was performed on March 4th, at swelling of buds, further sprayings were carried out on March 12, 17, and 21; and April 14, 1996.

**List of Treatments:**

1. 0.7% of test composition E in water with 0.025% of Triton X-100  
5 surfactant.
2. 1% of the above composition, as above.
3. Kocide™ (Copper hydroxide by Griffin Corp. USA.), 0.3% at bud swelling  
only;
4. Starner™ (Quinolinone compound, Sumitomo, Japan.), 0.15% for the rest  
10 of the five applications.
5. Control (no sprays).
6. Evaluation of results: On April 26, May 7, and May 21, 1996 counts were  
recorded of all infection centers typical to fireblight on the trees (including  
inflorescences, branches and fruit spurs).

15

**Table 4: Assessment of Fireblight Infection**

Treatments	Average Number of Necrotic Centers		
	April 26, 1996	May 7, 1996	May 21, 1996
1	14.25 A	68.50 AB	97.75 AB
2	14.00 A	35.25 A	72.25 B
3	5.25 A	27.25 A	66.25 B
4	31.50 A	91.75 A	134.75 A

The statistical analysis performed was Duncan Multiple Range Test at a degree of 5% significance. In table 1, assessment figures in a column accompanied by different letters are significantly different.

## 5 Laboratory Investigation of Response of Bacterial Cultures to Various Chelates

Two different bacteria strains were used throughout: one, *Erwinia carotovora* isolated from potato tuber soft rot, and *Pseudomonas spp.* isolated from soft rot of *Calla spp* tuber. Starter cultures of both strains (24 hours old) were used for inoculating liquid medium flasks each containing different concentrations of one of the test compositions described above. Growth of the bacteria in the medium was determined after 24 and 48 hours of incubation at 28°C by measuring the turbidity of the culture at 550 nm.

**Table 5a. *Erwinia* Growth in Culture as Affected By Test Compositions**

Figures denote turbidity of culture after 48 hours for increasing concentrations of test compositions in culture flasks.

Composition	% in Culture							
	Molarity of total metals	0.00	0.10	0.50	1.00	1.50	2.00	2.50
A	1.4	1.10	1.10	0.00	0.00	0.00	0.00	-
C	1.13	1.21	1.14	0.00	0.00	0.00	0.00	-
F	1.19	1.05	1.05	0.40	0.00	0.00	0.00	-
G	1.6	1.24	1.04	0.30	0.00	0.00	0.00	-
H	1.06	1.18	1.04	0.33	0.11	0.00	0.00	-
I	1.2	1.26	1.15	0.08	0.00	0.00	0.00	-

J	1.33	1.09	0.97	0.00	0.00	0.00	0.00	-
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Table 5b. *Pseudomonas* Growth in Culture as Affected By Test

## Compositions

- 5        Figures denote turbidity of culture after 48 hours for increasing concentrations of test compositions in culture flasks.

Composition		% in Culture						
		0.00	0.10	0.50	1.00	1.50	2.00	2.50
C	1.13	1.23	0.59	0.05	0.00	0.00	0.00	-
F	1.19	1.22	1.18	0.27	0.00	0.00	0.00	-
G	1.6	1.15	0.00	0.00	0.00	0.00	0.00	-
H	1.06	1.15	0.17	0.14	0.07	0.00	0.00	-
I	1.2	1.13	0.93	0.15	0.00	0.00	0.00	-
J	1.33	1.04	0.02	0.00	0.00	0.00	0.00	-

- The results of the above laboratory investigations show activity of all test compositions. Indication for specific activity of different compositions towards each of the two strains of bacteria are clearly noted. In addition, for each strain specific composition affect the bacteria to a different extent. Attention is drawn to test composition G, that was most concentrated. However in *Erwinia* it can be seen that for 0.5% concentration, test composition I was more effective although its molar concentration was lower. Composition G was much more effective inhibitor of growth of the *Pseudomonas* strain than it was of the *Erwinia* strain.

## Field Experiment 2: Phytotoxic Considerations

New, developing leaves of pear cultivar Spadona in early spring were sprayed with either 1% solution of the test solution C in water and surfactant as

above , whilst other trees were sprayed with test solution J in either 1% solution in water or in 0.6% concentration of the same. After three days, substantial scorching was observed in the leaves of the group sprayed with the 1% of solution J. Scorching also appeared in 0.6% of the same solution, in slightly less severe symptoms. The solution of 1% C in water exhibited almost no Phytotoxic  
5 symptoms, after the three day period and later on.



## CLAIMS

1. A composition for controlling fungal diseases which contains at least one type of chelating agent complexed with zinc ion and with at least another metal ion, whereas the chelating agent molecules are characterized in being either  
5 synthetic or natural molecules.
2. A composition for controlling fungal diseases which contains at least one type of chelating agent complexed with zinc ion and with at least another metal ion considered to have nutritive value, whereas the chelating agent molecules are characterized in being either synthetic or natural molecules.
- 10 3. A composition as in Claim 1 wherein the ingredients are in the form of aqueous solution.
4. A composition as in Claim 1 wherein the ingredients are in dry form, whereby the addition of measured amount of water is performed prior to application onto plants.
- 15 5. A method for protecting plants or parts of plants against fungal pests comprising applying to said plants a composition containing chelated zinc mixed with at least another chelated metal ion.
6. A composition for the control of bacterial diseases which contains at least one type of metal chelated by at least one type of chelating agent, whereas the  
20 chelating agent molecules are characterized in being any one or a combination of the following:
  - a) organic acid containing optionally more than one acidic group,

b) organic acid containing apart from the acidic group an additional functional group,

c) an alcohol or an amine optionally containing more than one functional groups,

5 d) an aromatic ring substitution product,

e) a synthetic chelating agent.

7. A composition as in Claim 6 wherein the ingredients are in the form of aqueous solution.

8. A composition as in Claim 6 wherein the ingredients are in dry form,  
10 whereby the addition of measured amount of water is performed prior to application onto plants.

9. A composition according to claim 6, wherein one metal is zinc.

10. A method for the control of bacterial infestations comprising:  
administration of a composition containing at least one chelated metal  
15 species of the group known to form chelates, whereas a combination of at least one type of metal with at least one type of chelating agents is used against a certain bacterial infestation, or as a precautionary measure before an infestation is expected to begin.

11. A method according to Claim 10, in which a bacterial strain is controlled  
20 better by a certain combination than by another combination.

12. A method according to Claim 10, in which Phytotoxic symptoms are less pronounced in using one type of mixture than in using another.

13. A method according to Claim 10, whereas at least one of the metals used alone or in combination is of a group known to be nutritious for plants or bacteria.
14. A method according to Claim 10, wherein the chelate composition is  
5 administered to plant roots, via trunks, for systemic effect, by injection, by drippers or by irrigation.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL00/00219

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(7) : A01N 25/00, 55/02, 59/16, 59/20 US CL : 424/405, 630, 639, 641, Dig. 6; 514/492, 494, 499 According to International Patent Classification (IPC) or to both national classification and IPC														
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Date of the actual completion of the international search 03 August 2000 (03.08.2000)		Date of mailing of the international search report <b>14 SEP 2000</b>												
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230		Authorized officer Frank Choi <i>Jerry J. Day for</i> Telephone No. (703) 308-1235												

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL00/00219

**Continuation of B. FIELDS SEARCHED Item 3: STN/CAS, WEST**

Search terms: citric acid, glycine, resorcinol, zinc, copper, manganese, chelate, fungal, bacterial

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